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TITLE: Efficacy of Gamma-glutamylcysteine (GGC) in Ischemia-reperfusion Injury

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14. ABSTRACT

Gamma-glutamycysteine (GGC) a precursor dipeptide for glutathione (GSH) was shown to inhibit oxidative stress in human umbilical vein endothelial cells (HUVEC). GGC protects against oxidative stress by alteration of gene expression of antioxidant defense independent of increased induction of endogenous GSH synthesis. In mixture studies looking at the effects of GGC with conjugated linoleic acid (CLA) in HUVEC, we found that the mixture protected against oxidative stress similar to treatment with GGC alone. We therefore suggest that GGC is likely a unique compound that exerts protection from oxidative stress by novel modulation of gene expression of antioxidant defense enzymes. During this 4-month period we examined the uptake of GGC using the everted gut sac isolated from rodents coupled with a sensitive high performance liquid chromatographic method (HPLC) using fluorimetric detection for measuring GGC levels. Findings suggest that GGC crosses the small intestine barrier and is GGC concentration depended in physiological range.

15. SUBJECT TERMS
Oxidative stress, antioxidants, glutathione, gamma-glutamycysteine.

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INTRODUCTION (from previous report):

Hemorrhagic shock is a leading cause of death in military combat and civilian trauma (Bellamy, 1984; Lieu, et al., 2004). Better understanding of the associated cellular biochemical changes that occur in ischemia-reperfusion (IR) injury can lead to efficacious therapies (Thomas et al., 2008). Therefore, we have been studying the feasibility of using gamma-glutamylcysteine (GGC) a dipeptide precursor for glutathione as a potential compound in modulating the oxidative stress associated with IR injury.

BODY:

Specific goal – Determine GGC doses to inhibit cellular oxidative stress and inhibit cellular death.

1. GGC inhibition of oxidative stress in human endothelial cells.

The objective of this study was to investigate the efficacy of GGC on GSH synthesis and oxidative stress in human endothelial cells, as a model for cellular oxidative stress. We found that GGC plays a role in GSH synthesis as a substrate for the antioxidant GSH and in modulating expression of proteins related to antioxidant defense as an inducer or suppressor.

2. Co administration of GGC and conjugated linoleic acid (CLA) in human endothelial cells.

The objective of this study was compared effects of co-administration of GGC and CLA with GGC alone on oxidative stress. We confirmed that GGC can substitute as an antioxidant for GSH without increasing GSH levels. Co-administration of CLA with GGC had differential effects depending on the dose of CLA. We believe that due to its ease of permeability through cell membranes, GGC could be used as an intra and intercellular therapeutic agent in oxidative stress-related injuries and diseases.

3. (New, June 30 – Oct 31, 2012) The objective was to assess the potential ability of GGC to be absorbed. Use of everted gut sac isolated from rats for studying the transport of GGC (5, 10 and 15 micromol/Liter) across the small intestine. Everted gut sacs were isolated from SD rats weighing approximately 250 g (El-Gorab et al, 1975; Barth et al., 1998; Mahmoud, 2004). Approximately 20 to 30% of the GGC was recovered and was GGC concentration dependent following 30 minutes of incubation of the suspended guts.

KEY RESEARCH ACCOMPLISHMENTS:

Two studies using human endothelial cells have been completed, peer reviewed and published. The studies indicate that GGC has efficacy in oxidative stress and suggest the potential usefulness of this compound in injuries and diseases associated with oxidative stress.

A sensitive method using high performance liquid chromatography (HPLC) and fluorimetric detection has been developed.

Preliminary studies suggest that GGC crosses the rat intestinal wall and is concentration dependent.

REPORTABLE OUTCOMES:

Published Abstracts

Nakamura, Y. K., Dubick, M. A., and **Omaye, S. T.** Gamma-Glutamylcysteine (GGC) inhibition of oxidative stress in human endothelial cells. Emerging Topics Section, Society of Toxicology Annual Meeting, 2011, Washington, D.C.

Nakamura, Y. K., Dubick, M. A., and **Omaye, S. T.** Effects of co-administration of gamma-glutamylcysteine (GGC) and conjugated linoleic acid (CLA) on oxidative stress in human endothelial cells. American Chemical Society Annual meeting, Denver, Colorado, 2011.

Published Peer-Reviewed Manuscripts

Nakamura, Y.K., Dubick, M.A., and Omaye, S. T. Gamma-glutamylcysteine inhibits oxidative stress in human endothelial cells. *Life Sciences* 90: 116-121, 2012.

Nakamura, Y.K., Dubick, M.A., and Omaye, S. T. Modulation of oxidative stress by γ-glutamylcysteine (GGC) and conjugated linoleic acid (CLA) isomer mixture in human umbilical vein endothelial cells. *Food and Chemical Toxicol* 60: 1854-1859, 2012.

CONCLUSION:

Although further studies are warranted to develop a better understanding about the efficacy of GGC supplementation under various conditions, GGC has potential as a therapeutic compound in modulation of oxidative stress. GGC appears to be absorbed in the gut and available to systemic circulation. Future studies will be directed at to better understand the mechanisms of action and eventual application of GGC in oxidative stress associated with ischemia reperfusion and hemorrhagic shock.

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APPENDICES:

1. Abstract for published manuscript in Life Sciences
2. Abstract for published manuscript in Food and Chemical Toxicology